Abstracts
of
Poster Session

Abstracts

Metabolism of Benzidine-, 3,3′-Dimethylbenzidine- and 3,3′-Dimethoxybenzidine-based Azo Dyes by Rat Intestinal Bacteria.

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Anaerobic bacterial suspensions isolated from rat intestinal contents reduced benzidine-, 3,3′-dimethylbenzidine- and 3,3′-dimethoxybenzidine-based azo dyes to potentially carcinogenic aromatic amines. The azo reduction products of Direct Black 38, Direct Red 2 and Direct Blue 15 formed by anaerobic intestinal bacteria were isolated by gas chromatography/chemical ionization mass spectrometry and identified by comparison of chromatographic and mass spectral properties to those of authentic standards. The results suggest that anaerobic intestinal bacteria are important in the metabolism of benzidine- and benzidine congener-based dyes in mammals and may play a significant role in the etiology of bladder cancer.

Metabolism of 1-Nitropyrene by Rat Intestinal Bacteria.

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The nitrated polycyclic aromatic hydrocarbon 1-nitropyrene is an environmental contaminant, a bacterial mutagen, and has recently been shown to be carcinogenic in male rats. We have demonstrated previously that metabolic reduction of the nitro function results in the formation of an electrophilic-DNA binding species and, therefore, have investigated the metabolism of 1-nitropyrene by anaerobic rat intestinal bacteria. Suspensions of bacteria (1×10^9 cells/mL) were incubated for 24 hr anaerobically in media containing 8 μM [4,5,9,10-^{3}H]-1-nitropyrene (117 mCi/mmole). After 1 hr, all of the [^{3}H]1-nitropyrene was metabolized to a compound that co-eluted with 1-aminopyrene upon analysis by high performance liquid chromatography. Subsequent mass spectral analysis confirmed this identification. The metabolism of 1-nitropyrene to 1-aminopyrene was not accompanied by the formation of *N*-acetyl-1-aminopyrene, a metabolite reported to be formed in *Salmonella typhimurium*. These results indicate that rat intestinal anaerobic bacteria have the capability to reduce 1-nitropyrene to 1-aminopyrene, and suggest that anaerobic intestinal microflora may play a significant role in the *in vivo* reduction and carcinogenicity in mammals of nitro polycyclic aromatic hydrocarbons.

Induction of Hepatic Microsomal Oxidases by 3,3′-Dichlorobenzidine in the Rat.

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Intraperitoneal administration of 3,3′-dichlorobenzidine (DCB) to adult male rats, resulted in a dose- and vehicle-dependent induction of hepatic microsomal cytochrome P-448. Of the three cytochrome P-448-mediated oxidations studied [ethoxycoumarin, *p*-nitrophenetole *O*-deethylase and aryl hydrocarbon hydroxylase (AHH)], only AHH was lower in microsomes from DCB-pretreated rats (DCB-m) than in microsomes from 3-methyl-cholanthrene-pretreated rats (MC-m). However, NADPH-dependent covalent binding of ^{14}C-benzo(a)pyrene to hepatic microsomal protein was higher in DCB-m than in microsomes from untreated rats, was inhibited by 7,8-naphthoflavone but was stimulated by SKF-525A and by carbon monoxide. NADPH-dependent peroxidation of microsomal lipids was stimulated in DCB-m; this *in vitro* activity was inhibited by added DCB. The data suggest the presence of multiple pathways for the oxidative metabolism of DCB and that the catalytic activities of cytochrome P-448 induced by DCB may contribute to the hepatocarcinogenicity of the chemical.

Oxidation of 3,5,3′,5′-Tetramethylbenzidine by Peroxidases: Optical and Electron Spin Resonance Investigations.

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The activation of benzidine to an ultimate carcinogenic metabolite is believed to involve enzymatic oxidation. Although the oxidation of benzidine to colored products by the horseradish peroxidase/H_2O_2 system is well-known, the products have not been characterized unambiguously. In our studies, we have used the benzidine derivative 3,5,3′,5′-tetramethylbenzidine (TMB) as a model substrate. Two colored products are obtained: a blue product (½ mole peroxide per mole TMB) and a yellow product (equimolar peroxide). The yellow product is the diimine derivative of TMB (2-electron oxidation product). The blue product is a charge-transfer complex composed of one molecule of TMB and one molecule of the diimine. The charge-transfer complex is in equilibrium with the cation free radical ($TMB^{+\cdot}$). The free radical was detected by electron spin resonance spectroscopy. Computer simulation of the spectra observed in H_2O and D_2O buffers permitted the assignment of hyperfine splitting constants.

The prostaglandin synthase/arachidonic acid system catalyzes the co-oxidation of a variety of carcinogens. TMB is oxidized to a free radical in incubations containing ram seminal vesicle microsomes (as a source of prostaglandin synthase) and arachidonic acid, at pH 7.0. The ESR spectrum of the radical is identical to that obtained with horseradish peroxidase/H_2O_2, but the products are less stable at neutral pH.

Oxidation of Benzidine by Peroxidases: Chemical Studies and Biological Implications.

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Benzidine and its derivatives (such as tolidine and dianisidine) are oxidized by the horseradish peroxidase/H_2O_2 system. The products are the benzidine cation free radical (identified by electron spin resonance spectroscopy), the benzidine diimine (yellow-colored, identified by optical spectroscopy), and the blue-colored charge-transfer complex of benzidine and the diimine. These products are analogous to those formed by the oxidation of 3,5,3′,5′-tetramethylbenzidine, but the benzidine oxidation products are short-lived, even at acid pH.

The oxidation products are rapidly reduced back to the parent compound following the addition of stoichiometric amounts of ascorbic acid (vitamin C).

The diimine derivative of benzidine reacts rapidly with a variety of phenol derivatives, including butylated hydroxyani-

sole (BHA), epinephrine, and serotonin. The resulting adducts are violet-colored and stable. We have characterized these adducts, using 2,6-dimethylphenol as a model compound. Mass spectrometry and NMR spectroscopy were used to determine the structure of the benzidine/2,6-dimethylphenol adduct. The adduct is an indophenol, analogous to the product formed from the reaction of phenols with Gibbs' reagent (2,6-dichloroquinone chloroimine).

The benzidine nitrenium ion, postulated to be the ultimate carcinogenic metabolite of aromatic amines, may be drawn as a resonance structure of the (monoprotonated) diimine.

Cooxidation of Benzidine by Horseradish Peroxidase and Subsequent Formation of Possible Thioether Conjugates of Benzidine. JOHN R. RICE and PETER T. KISSINGER, *Department of Chemistry, Purdue University, West Lafayette, IN 47907.*

Combined liquid chromatographic and electroanalytical techniques have been applied to a study of a possible minor metabolic pathway of toxic, easily-oxidized aromatic compounds. As a representative model, the human bladder carcinogen benzidine was found to be converted by horseradish peroxidase and H_2O_2 to an intermediate which reacts with thiol-containing nucleophiles to give rise to a pattern of products which are identical to those produced by reaction of the two-electron oxidation product of benzidine, 4,4′-biphenoquinonediimine, under identical conditions at physiological pH. The properties of the major reaction product are dependent on the identity of the thiol employed and are consistent with those expected for a ring-(S)-thioether conjugate of benzidine. These results may be indicative of a previously unrecognized avenue of benzidine metabolism, and could have important implications about the activation of many other toxic organic molecules by oxidoreductase enzymes. Several examples of results obtained from solution studies, tissue preparations, and whole animal studies by this analytical approach will be displayed.

Oxidative Metabolism and Macromolecular Binding of Carcinogenic Primary Arylamines Catalyzed by Prostaglandin Synthetase. CLAY B. FREDERICK, CONSTANCE C. WEIS, and FRED F. KADLUBAR, *National Center for Toxicological Research, Jefferson, AR 72079* and TERRY V. ZENSER, *St. Louis VA Medical Center, St. Louis, MO 63125.*

Oxidative metabolism of the carcinogens, 2-naphthylamine (2-NA), benzidine (BZ), 4-aminobiphenyl (4-ABP), and 2-aminofluorene (2-AF), has been studied *in vitro* with a prostaglandin synthetase preparation from ram seminal vesicles. Very rapid and extensive metabolism was evident in all cases, as measured by loss of substrate. The principal metabolites of [^{3}H]2-NA, [^{3}H]4-ABP, and [^{3}H]2-AF were found to be nitrosoarenes and their subsequent condensation products. Significant binding of the tritiated metabolites to protein was observed. Metabolite formation and protein binding were dependent on arachidonic acid and were inhibited by the prostaglandin synthetase inhibitor indomethacin. A low but significant level of carcinogen-DNA binding was also detected with added calf thymus DNA. These *in vitro* observations indicate that prostaglandin synthetase-mediated metabolism occurs with a variety of primary arylamines to produce *N*-oxidized products which may react with cellular macromolecules.

Lipoxygenase-Peroxidase Activation of *N*-Hydroxy-2-Acetylaminofluorene by Rat Mammary Gland Parenchymal Cells. P. K. WONG, S. L. NANK, and R. A. FLOYD, *Oklahoma Medical Research Foundation, Oklahoma City, OK 73104.*

N-Hydroxy-2-acetylaminofluorene (N-OH-AAF) can be activated into two more potent carcinogens, namely, 2-nitrosofluorene (NOF) and *N*-acetoxy-2-acetylaminofluorene (N-OAc-AAF), by rat mammary cells via a nitroxyl free radical form of the carcinogen. The detection of N-OAc-AAF and NOF as major products by high pressure liquid chromatography (HPLC), the presence of nitroxyl free radical as detected by electron spin resonance spectroscopy (ESR) and the inhibition produced by peroxidase inhibitors such as ascorbate and *p*-aminophenol strongly suggested the involvement of peroxidative oxidation process. Presence of peroxidase in the mammary cells is further confirmed by light microscopic staining techniques. Ram seminal vesicle microsomes also activate N-OH-AAF to NOF. Unlike the prostaglandin synthetase, mammary cell enzymes are not affected by indomethacin or aspirin, while lipoxygenase inhibitors, 5,8,11,14-eicosatetraynoic acid (ETYA), gossypol and Fe(III) chelators such as dithizon inhibit the activation process. Externally added arachidonate enhances the cell activity. The hydroperoxides generated from exogenous and endogenous arachidonate by the cell lipoxygenase are thought to be the substrate for the peroxidase activation mechanism. Activity of intact cell isolated from mammary gland with the lymph nodes is higher than those with the lymph nodes removed. Sonicated and frozen cell preparations have highest activities. Inhibition studies based on the measurement of N-OAc-AAF seem to indicate that an additional possible mechanism may be involved in generating this product. The nature of this possible process is presently under investigation. Mammary cells isolated from rats on selenium-supplemented diet are less active than cells from animals on selenium-deficient diet indicating possible correlation between cell lipoxygenase and peroxidase activities and mammary carcinogenesis.

Microsomal Metabolism of 4-*N,N*-Diacetylaminobenzo[a]pyrene, an Aromatic Amide Derived from a Potent Polycyclic Aromatic Hydrocarbon. MING W. CHOU, DWIGHT W. MILLER and PETER P. FU, *National Center for Toxicological Research, Jefferson, AR 72079.*

Mechanistic studies with aromatic amines have been focused primarily on compounds derived from noncarcinogenic polycyclic aromatic hydrocarbons (PAHs), such as fluorene, naphthalene, anthracene, biphenyl and phenanthrene. Little is known about the biological activities of aromatic amines which are formed from carcinogenic PAHs, such as benzo[a]pyrene (BP). With compounds of this type, multiple bioactivation pathways, occurring both through the amino functional group and the aromatic ring, are possible. We report here the aerobic rat liver microsomal metabolism of 4-*N,N*-diacetylamino-BP (4-NAc_2BP). This compound was previously found to be much more mutagenic than BP or 2-aminofluorene in Ames' *Salmonella typhimurium*/microsome reversion assay, and its mutagenicity decreased when tested in the presence of the deacylase inhibitor, paraoxon. The metabolites were purified by high performance liquid chromatography and characterized by physicochemical methods including UV-VIS absorption, mass and NMR spectroscopy. 9-Hydroxy-4-NAc_2BP, 1,9-

dihydroxy-4-NAc$_2$BP, *trans*-7,8-dihydrodiol-4-NAc$_2$BP, and 5-hydroxy-4-N-acetylamino-BP were identified as major metabolites. The metabolite *trans*-7,8-dihydrodiol-4-NAc$_2$BP may be a precursor of 7,8-dihydrodiol-9,10-epoxide-4-NAc$_2$BP, a possible activated metabolite. Thus, this result and the results of mutagenicity assay with and without paraoxon suggest that both oxidation of the amino (or amide) group and oxidation of the aromatic ring are involved in the metabolic activation pathways.

Kinetics of *N*- and *C*-Hydroxylations of 2-Acetylaminofluorene in Rat Liver Microsomes.

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2-AAF undergoes cytochrome P-450 dependent oxidation via *N*- and *C*-hydroxylations. *N*-Hydroxylation is considered to be the initial and obligatory step in the metabolic activation of 2-AAF to a carcinogen, while *C*-hydroxylations appear to be detoxification pathways. For an estimate of the significance of these pathways in the metabolism of AAF, a kinetic study was undertaken using rat liver microsomes and substrate concentrations ranging from 0.02 to 200 μM. *N*-Hydroxylation accounted for approximately 50% of the metabolites at low substrate concentrations (<0.2 μM) but less than 5% at concentrations of AAF above 10 μM. The K_m and V_{max} for this reaction were 0.04 μM and 6.0 pmole/min/mg, respectively. While *N*-hydroxylation was best described by a single enzyme system (single catalytic site), two enzyme kinetics (two catalytic sites) were observed for 1-, 3-, 5- and 7-hydroxylations. For example, Eadie-Scatchard plots revelaed a high affinity (K_m = 0.07 μM) low capacity (4.87 pmole/min/mg) enzyme and a low affinity (K_m = 99 μM) high capacity (1289 pmole/min/mg) enzyme for the 7-hydroxylation of AAF. These data indicate that more than one form of cytochrome P-450 is involved in each of the *C*-hydroxylations, whereas only one form catalyzes the *N*-hydroxylation.

Metabolism and Genotoxicity of a Series of 2-Acetylaminofluorene and Phenacetin Derivatives.

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N-Hydroxylation is believed to be a factor in the activation of 2-acetylaminofluorene (AAF) and phenacetin (P) to carcinogenic metabolites. The present studies involved comparison of *N*-hydroxy derivatives of P and AAF in guinea pig and rat liver metabolic activation systems. Guinea pig liver microsomes were subjected to gel filtration on Sephacryl S-200. Analysis of the column fractions gave the following results. Assays for *N*-hydroxy-AAF (N-OH-AAF) deacylation and *N*-hydroxy-P (N-OH-P) binding to tRNA revealed a single peak of activity (Peak I). N-OH-AAF or *N*-hydroxy-formylaminofluorene (N-OH-FAF) binding activity, however, was catalyzed by two peaks (I and II), peak II having a smaller molecular weight. HPLC analysis of metabolism by peak I revealed that the major metabolites were the corresponding nitroso derivatives, either nitrosofluorene for N-OH-AAF and N-OH-FAF, or nitrosophenetole for N-OH-P, which would result from the spontaneous oxidation of arylhydroxylamines. Relatively little metabolism of N-OH-P, N-OH-AAF, or N-OH-FAF occurred in the presence of peak II. Unscheduled DNA synthesis (UDS) in cultured rat liver cells was also examined. AAF, N-OH-AAF, and nitrosofluorene were all active in the 10^{-6} *M* to 10^{-4} *M* range, resulting in 30-50 grains per nucleus when ^{3}H-thymidine incorporation was measured autoradiographically. Of the corresponding P derivatives, however, only N-OH-P was active, inducing 5-30 grains per nucleus in the 10^{-6} *M* to 10^{-4} *M* range. The data in these guinea pig and rat liver systems indicate that the hydroxamic acids of AAF and P are activated to nucleic acid-binding and genotoxic metabolites by mechanisms which are only partially explained by deacylation.

Metabolism of *N*-Hydroxy-2-acetylaminofluorene by Guinea Pig Liver Microsomes.

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The guinea pig is resistent to the hepatocancerogenic effect of aromatic amides like 2-acetylaminofluorene (2-AAF). It has for long been assumed that this resistance was due to the inability of the liver tissue of this animal species to *N*-hydroxylate 2-AAF. This hypothesis was however contradicted by the conclusions of various reports demonstrating that guinea pig liver 9,000*g* supernatants activate 2-AAF as a mutagen in the Ames test.

The present communication offers experimental evidence to the following. Guinea pig liver microsomal mixed-function oxidases *N*-hydroxylate both 2-AAF and 2-AF. Guinea pig liver microsomal arylamide (2-AAF) *N*-hydroxylase is quantitatively (V_{max}) but not qualitatively (K_M) equivalent to the rat liver microsomal enzyme. Guinea pig liver microsomal arylamine (2-AF) *N*-hydroxylase is the highest *N*-hydroxylating enzymic activity of all tested species (rat, hamster, mice). It has also the highest enzymic affinity. Those enzymic activities are only slightly, if at all, induced by pretreatment with polycyclic aromatic hydrocarbon. Guinea pig liver microsomes have a specific enzyme activity which metabolize *N*-hydroxy-2-AAF, to the inactive, nongenotoxic C7 hydroxy metabolite. Both the 7-fluoro- and 7-iodo-2-acetylaminofluorene are very efficiently activated by guinea pig liver microsomes. All these experimental data clearly suggest that the resistance of the guinea pig to the hepatocarcinogenic effects of 2-AAF and 2-AF is due mainly to the ability of that tissue to metabolize the active *N*-hydroxyl metabolite to an inactive ring hydroxylated product. The relatively low affinity of the *N*-hydroxylating enzyme is another factor which explains the difference between the result of *in vivo* (carcinogenicity) and *in vitro* (mutagenicity) experiments.

Enzymology of Hepatic Microsomal *N*-Hydroxylases.

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The primary, most probable rate-limiting step in the metabolic activation of mutagenic and cancerogenic aromatic amines and amides is their mixed-function oxidase-dependent *N*-hydroxylation. The mixed-function oxidase activity is cytochrome P-448-dependent but not enzymologically completely identical to benzo(a)pyrene monooxygenase which is classically measured as the aryl hydrocarbon hydroxylase. The present communication compares the enzymatic properties of the liver microsomal arylamide and arylamine *N*-hydroxylases of various animal species (rat, hamster, mouse, dog, guinea pig, monkey, rabbit, and human). It analyzes the effects of

various pretreatments *in vivo* (3-methylcholanthrene, 2-AAF) on the enzymatic properties both in terms of induction (increased V_{max}) and modification (change in K_M). It reports on the *in vitro* effects of various effectors of the mixed-function oxidases like SKF-525A, metyrapone, imidazole derivative, polycyclic aromatic hydrocarbons, benzoflavone and position isomers of 2-AAF. All these data are discussed in relation to the properties of both arylamine and arylamide *N*-hydroxylases as a mixed function oxidase activity, the role of this primary activation step in the mutagenicity of 2-AAF and 2-AF, and the importance of the enzymatic interactions between *N*-hydroxylation and C-hydroxylation in explaining differences in species susceptibility to the genotoxic effects of 2-AAF and 2-AF.

Effects of Pretreatment by 3-Methylcholanthrene on the Enzymic Properties of Liver Microsomal *N*-Hydroxylase. C. RAZZOUK, M. BATARDY, and M. ROBERFROID, *Unit of Biochemical Toxicology and Cancerology, Université Catholique de Louvain, B-1200 Brussels, Belgium.*

Microsomal *N*-hydroxylase is the primary, most probably rate-limiting step in the metabolic activation of mutagenic and cancerogenic arylamines and arylamides. The enzymic activity is cytochrome P-448-dependent. It is thus inducible by 3-methylcholanthrene (3-MC). Using rat, hamster and various strains of mice (NMRI, C57BL6 and DBA2) we have analyzed the effects of pretreatment by 3-MC on the enzymic properties of liver microsomal *N*-hydroxylases. Except for DBA2 mice, all enzymic activities are highly inducible by 3-MC, and the inducing effect is inversely related to the initial level of activity. As compared to benzo(a)pyrene hydroxylase, the induction of arylamide *N*-hydroxylase is much larger in the rat liver. 3-MC pretreatment also modifies kinetic properties of the liver microsomal N-hydroxylases, causing either a decrease (hamster, NMRI mouse), an increase (rat, C57BL6 mouse) or no change (DBA2 mouse) in its apparent K_M value. C57BL6 mouse appears to be a peculiar animal strain with regard to the effect of 3-MC on the enzymic properties of liver microsomal *N*-hydroxylase. The value of the apparent K_M of that induced enzyme is indeed dependent upon the concentration of microsomal proteins in the incubation medium. It is activated, *in vitro*, by micromolar concentrations of both paraoxon and 8-hydroxyquinoline. Moreover, like the guinea pig liver preparation, 3-MC induced C57BL6 liver microsomes very actively metabolize *N*-hydroxy-2-acetylaminofluorene. All these biochemical data are discussed in correlation with the mutagenicity of 2-AAF in the Ames test in the presence of liver microsomes from controls or the 3-MC induced rat, hamster and mouse (NMRI, C57BL6 or DBA2).

Rapid Production, Isolation and Biochemical Analysis of Liver Preneoplastic Cells. M. LANS, J. DE GERLACHE, H. TAPER, V. PREAT and M. ROBERFROID, *Unit of Biochemical Toxicology and Cancerology, Université Catholique de Louvain, B-1200 Brussels, Belgium.*

Most experimental procedures developed to produce preneoplastic lesions in rat liver require long protocols before the nodular lesions are sufficiently developed to allow biochemical determination. In the present work, the procedure of Solt and Farber for the production of preneoplastic lesions was associated with the subsequent administration of a promoter (phenobarbital). Our results show that, in such a protocol, 15 weeks after the initiation dose of diethylnitrosamine, more than 50% of the liver volume is occupied by preneoplastic nodules. These nodules display the usual histological, histochemical and biochemical features. As described previously, the cells of these nodules have been isolated and biochemically analyzed. In particular, drug-metabolizing enzymic activities in the preneoplastic cells have been compared both qualitatively and quantitatively with the same enzyme activities in the surrounding parenchymal cells. Nodular cytochrome P-450 content was decreased. Moreover, even though several mixed function oxidases activities were proportionately reduced, 2-acetylaminofluorene (2-AAF) *N*-hydroxylase was much more affected than the other enzymes (benzopyrene hydroxylase, aldrin monooxygenase). Such a specific impairment of the arylamide activating enzyme might explain the effectiveness of 2-AAF in the selection procedure developed by Solt and Farber and the resistance of preneoplastic cells to its anti-proliferative effect. However, despite this loss of mixed-function oxidase activities, preneoplastic nodular hepatocytes responded readily to *in vivo* induction by 3-methylcholanthrene. Those results indicate that, at this stage of the transformation process, nodular cells still respond to inductive stimuli.

Metabolism of 2-Acetylaminofluorene and Benzidine by Short-Term Organ Cultures of Rat and Human Bladder Epithelium. B. P. MOORE and R. M. HICKS, *School of Pathology, Middlesex Hospital Medical School, London, W1P 7LD, U.K.*

2-Acetylaminofluorene (AAF) induces urinary bladder tumors in rodents, and benzidine (BZ) induces similar tumors in man. Short-term organ cultures of rat and human bladder were found to metabolize both these carcinogens to proximate carcinogenic intermediates. It is believed that unmetabolized or partially metabolized arylamine carcinogens can be released from certain dye materials into the urine. In theory, urothelial cell-mediated metabolism of such arylamines could result in the induction of tumors.

Rat Liver Microsomal Metabolism of Reduced Michler's Ketone (RMK) *In Vitro* and Disposition and Metabolism *In Vivo* in Rats. R. F. STRUCK, D. J. MCCARTHY, S. E. ENKE, T. -W. SHIH, W. J. SULING, and D. L. HILL, *Southern Research Institute, Birmingham, AL 35255.*

Incubation of ^{14}C-RMK with a 9000*g* fraction of livers from male Osborne-Mendel rats pretreated with phenobarbital, chloroform extraction of the incubation mixture, and TLC analysis of the extract along with synthetic standards yielded only demethylated products in the following percentages: RMK (29.3%), *sym*-didemethyl-RMK (5.3%), *unsym*-didemethyl-RMK (44.7%), tridemethyl-RMK (11.8%), tetrademethyl-RMK (6.9%), unidentified at origin (2.0%).

Tissue distribution studies in male Osborne-Mendel rats following intraperitoneal injection of ^{14}C-RMK (200 mg/kg) indicated the following major sites of radioactivity: after 1 hr, liver, fat, stomach, small intestine; after 3 hr, liver, fat, small intestine; after 24 hr, liver, fat, large and small intestine. A single rat given ^{14}C-RMK (164 mg/kg) excreted, after 24 hr, 15.2% of the radioactivity in urine, 5.1% in feces, but none in expired air; 73% was retained in the carcass.

The major urinary metabolite was identified as *N,N'*-diacetyl-4,4'-hydroxymethylendianiline and accounted for 36% of the urinary radioactivity; 43% was present as RMK.

Neither RMK nor any of the microsomal metabolites were direct-acting mutagens in the standard *S. typhimurium* assay, but all were activated to mutagens upon incubation with a 9000*g* fraction of mouse liver, the number of revertants being proportional to the extent of demethylation. The results suggest that the methyl groups of RMK are not required for conversion to a mutagen.

Hepatic Macromolecular Covalent Binding and Intestinal Disposition of 2,6- and 2,4-Dinitrotoluene in Fischer-344 Rats.

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Technical grade dinitrotoluene (DNT) (75.8% 2,4-DNT, 19.5% 2,6-DNT, 4.7% other isomers) is hepatocarcinogenic in Fischer-344 rats. 2,6-DNT is more genotoxic than 2,4-DNT, and it produces a positive response for initiation in *in vivo* hepatic initiation/promotion systems while 2,4-DNT does not. The purpose of this study was to compare the nature and extent of covalent binding to hepatic macromolecules after administration of ^{14}C-2,4-DNT or ^{14}C-2,6-DNT. Male Fischer-344 rats were given 10 or 35 mg ^{14}C-2,4-DNT or ^{14}C-2,6-DNT/kg by gavage and killed at various times thereafter. Livers were removed, weighed and homogenized. The homogenate was separated into DNA, RNA and protein enriched fractions which were then counted to determine ^{14}C covalently bound. Sections of small intestine and cecal contents were analyzed for total radioactivity and DNT metabolites. An initial peak (1-2 hr) of radioactivity appeared in the liver after each isomer, but no significant covalent binding occurred at that time. Appearance of 2,4- or 2,6-dinitrobenzyl alcohol glucuronide and the corresponding aglycones in the intestine was followed by a second peak of total hepatic ^{14}C at 12 hr. At this time covalent binding to DNA, RNA and protein became significant and persisted at least until 96 hr after the dose. Neither isomer showed a specificity for a particular macromolecule, but covalent binding to DNA, RNA and protein was 2-5 times greater after 2,6-DNT than after 2,4-DNT. These data correlate with the relative genotoxicities of the two isomers and strongly support a role for enterohepatic circulation in the formation of DNT metabolites capable of covalently binding to hepatic macromolecules.

Formation of Free Radicals by the Interaction of Aromatic Nitro Compounds with Biochemical Systems.

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The biological activities of some antimicrobials, mutagens, and carcinogens may be due to reduction of the nitro group by nitroreductases. This proceeds by sequential one-electron reduction steps. The first step leads to the nitroradical anion which can transfer its electron to oxygen with concomitant formation of superoxide radical and the parent nitro compound; therefore, the presence of oxygen usually inhibits nitroreductase activity. We have studied the formation of free radicals from nitro compounds (e.g., 4-nitroquinoline-*N*-oxide (NQO), *p*-nitrobenzonitrile, chloramphenicol (CP) and metronidazole) with biochemical reducing systems incorporating cytochrome P-450 reductase, xanthine oxidase or rat liver microsomes. Free-radical formation was monitored by EPR spectroscopy in the presence as well as the absence of spin-traps. The formation of ·OH radical, as the spin adduct of 5,5-dimethylpyrroline-*N*-oxide (DMPO), was seen in aerobic incubations of nitro compounds with the different reducing systems. In some instances, it was also possible to trap the superoxide free radical. When the incubations were carried out under anaerobic conditions, the formation of nitroradical anions was observed. When NQO or chloramphenicol was incubated with rat liver microsomes and an NADPH generating system composed of NADP, glucose-6-phosphate and glucose-6-phosphate dehydrogenase under anaerobic conditions, the nitro group was reduced as judged by ultraviolet spectroscopy. The incubation mixture was presumed to contain the hydroxylamine derivative and potassium ferricyanide was added in order to convert the hydroxylamine to the corresponding nitroso derivative. This presumption was shown to be correct when addition of 2,3-dimethylbut-2-ene to the oxidized mixture resulted in the trapping of the nitroso compound with concomitant generation of a fairly stable nitroxyl which was easily detected by EPR spectroscopy.

Thiols Protect against the Mutagenicity of 2-Nitrosofluorene toward *Salmonella typhimurium* TA 98.

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The metabolic conversion of 2-acetylaminofluorene (AAF) to potent mutagenic and carcinogenic species is well known. One of the metabolities of AAF is 2-nitrosofluorene (NOF) which is highly mutagenic to *Salmonella typhimurium* TA 98 in the Ames assay, even in the absence of S-9 liver microsomal system. Thiols (sulfhydryl compounds) are known to react with nitroso compounds such as NOF. Therefore, we tested the effect of thiols such as reduced glutathione (GSH), cysteine (CySH), 1,4-butanedithiol, 1,2-ethanedithiol, ethanethiol and mercaptoethanol on the mutagenicity of NOF in the Ames assay with *Salmonella typhimurium* TA 98. All of the thiols tested protected the bacteria from mutagenesis by NOF. In a separate set of experiments, the bacteria were incubated with GSH or CySH and subsequently washed twice with physiological saline to remove extracellular thiol. The pretreated bacteria were protected from the mutagenic action of NOF. This suggests that intracellular pools of sulfhydryl compounds may afford protection against mutagenesis by NOF. Experiments designed to deplete the sulfhydryl content of bacteria by treatment with 1,1'-azobis(*N,N*-dimethylformamide) (diamide) or *N*-ethylmaleimide resulted in extensive cell killing. Catalase was included in the mutagenesis assay systems in order to prevent toxicity of hydrogen peroxide which is produced during autooxidation of thiols.

Modulation of Aromatic Amine Mutagenicity in *Salmonella typhimurium* Cocultured with Monolayers of Isolated Rat Hepatocytes.

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Hepatocytes isolated from Wistar rats by collagenase perfusion and cultured as monolayers were used to study the mutagenic activation of 2-acetylaminofluorene (AAF), 2-aminofluorene (AF) and *N*-hydroxy-2-acetylaminofluorene (N-OH-AAF). All three compounds were converted to mutagens excreted into the incubation medium, with N-OH-AAF>AF>AAF. Various modulators of aromatic amine metabolism were tested and compared to effects in subcellular

activation systems. Metyrapone inhibited AAF, but not AF or N-OH-AAF mutagenicity, whereas α-naphthoflavone inhibited AF, but not AAF or N-OH-AAF mutagenicity. Paraoxon totally blocked AAF mutagenicity, but only inhibited AF and N-OH-AAF mutagenicity by 20%. Ascorbate, on the other hand, increased AAF, AF and N-OH-AAF mutagenicity by 65, 81, and 26%, respectively. Lowering of cellular glutathione (GSH) levels with diethyl maleate and methionine sulfoxime did not affect AAF, AF or N-OH-AAF mutagenicity, whereas GSH addition to the incubation medium was inhibitory. Inhibition of cellular UDPGA formation with galactosamine increased AAF and AF mutagenicity, whereas inhibition of sulfation reactions with pentachlorophenol in a sulfate-free medium was without effect. The present study shows that both similarities and differences were found when results from the whole cell system were compared with those from subcellular systems.

Lack of Comutagenicity by Norharman and Other Compounds on Revertants Induced by Photolabeling with 2-Azido-9-fluorenone Oxime. W. E. WHITE, JR., and S. G. ROCK, *Department of Biochemistry, University of Alabama in Birmingham, Birmingham, AL 35294.*

The photolabeling technique has been used previously to induce mutations in bacteria and yeast as well as transformations in 10T 1/2 cells. As a tool for investigating particular aspects of mutagenesis, it has definite advantages because the active intermediates are generated by photolysis rather than by metabolism.

It has been suggested that some compounds, particularly harman and norharman, are comutagenic because they bind to DNA and alter the conformation, thereby facilitating the formation of critical adducts. The photolabeling technique permits testing this hypothesis because the effects of the suspected comutagen resulting from metabolism are eliminated.

Salmonella typhimurium TA 1538 was incubated in suspension with a 10^{-7} solution of 2-azido-9-fluorenone oximes containing varying concentrations of harman, norharman, ethidium, bile acids and some chlorinated dibenzo dioxins, including the 2,3,7,8-tetrachloro isomer. The bacteria were photolyzed and plated. No compound tested was comutagenic. At high doses (10^{-5}-$10^{-4}M$) the number of revertants was reduced with some compounds.

These results do not support the hypothesis that alteration of mutagen-DNA binding is a mechanism of comutagenesis; however, these studies cannot rule out this mechanism for other mutagens and/or other comutagens.

Susceptibility of Hepatocytes from Rapid and Slow Acetylator Rabbits to the Genotoxicity of Aromatic Amines. CHARLENE A. MCQUEEN and GARY M. WILLIAMS, *American Health Foundation, Valhalla, NY 10595.*

Rabbits and humans have genetically determined polymorphic differences in acetylation rates such that individuals can be separated into rapid or slow acetylators. The liver is a major organ of *N*-acetylation of xenobiotics, and rabbit hepatocytes in primary culture express and maintain the *in vivo* polymorphism. Hepatocytes were isolated from rabbits of both phenotypes, in order to investigate the relationship between the acetylator polymorphism and susceptibility to the genotoxic effects of aromatic amines. DNA repair, determined by autoradiography, was used as an indicator of genotoxicity, i.e., DNA damage. Rapid acetylator hepatocytes were more sensitive to the genotoxic effects of 2-aminofluorene than were slow acetylators. The rapid phenotype was also more susceptible to the genotoxic effect of benzidine, with maximum DNA repair occurring at 10^{-5} *M*. For slow acetylators, a dose of 10^{-3} *M* was necessary to elicit repair. The quantitative response in the rapid acetylator at 10^{-5} *M* was greater than that observed in the slow acetylator even at the 100-fold higher concentration. Preliminary evidence for methylenebis-2-chloroaniline and 4-aminobiphenyl indicated genotoxicity but no clear correlation with acetylator phenotype. 2-Naphthylamine was not genotoxic to either phenotype. With the acetylated derivatives of 2-aminofluorene, 2-acetylaminofluorene or *N*-hydroxy-2-acetylaminofluorene, no differences in DNA repair occurred in either phenotype. Thus, the demonstration of phenotype-dependent differences in genotoxicity provides evidence for the role of the acetylator polymorphism in determining susceptibility to the effect of aromatic amine carcinogens.

The Role of the *uvr* Genes in *E. coli* for Repair of 2-Acetylaminofluorene and 2-Aminofluorene DNA Adducts. M-S. LANG and M. W. LIEBERMAN, *Department of Pathology, Washington University School of Medicine, St. Louis, MO 63110* and C. M. KING, *Department of Chemical Carcinogenesis, Michigan Cancer Foundation, Detroit, MI 48201.*

In order to study the biological effect of chemical carcinogen-induced DNA damage and the role of different *E. coli uvr* genes in its repair, we have modified bacteriophage ϕX174 RF DNA with *N*-acetoxy-2-acetylaminofluorene (NA-AAF) and *N*-hydroxyaminofluorene (N-OH-AF) *in vitro* and used it for transfection of $CaCl_2$-treated different *E. coli C* strains with a nearly isogenic background. High performance liquid chromatography analysis showed that NA-AAF treatment produces predominantly *N*-(guanin-*C8*-yl)-*2*-acetylaminofluorene (85%) and N-OH-AF treatment produces solely *N*-(guanin-*C8*-yl)-aminofluorene (~100%). The results of transfection of these two kinds of modified DNA in different *E. coli* strains indicates that repair of G-C8-AAF is similar to the repair of pyrimidine dimers in requiring the *uvrA, B* and *C* gene products; however, repair of G-C8-AF requires only the *uvrC* gene product. Furthermore, G-C8-AF is about ten times less lethal than G-C8-AAF. These data suggest that pyrimidine dimers and some bulky chemical adducts are not repaired in precisely the same way by the *uvr* system and that the *uvrC* gene product can function either collectively with *uvrA* and *uvrB* gene products or independent of them for the repair of different DNA damage.

Modification of Guanosine by the Hydroxylamine Derivatives of 2-Nitroimidazoles. A. J. VARGHESE and G. F. WHITMORE, *Ontario Cancer Institute, 500 Sherbourne Street, Toronto M4X 1K9, Ontario, Canada.*

Misonidazole, after reduction to the hydroxylamine derivative, was found to react with guanosine in aqueous solution at pH 7. The guanosine product was isolated and was assigned a structure having a new five-membered ring with a -CHOH-CHOH- linkage between the N1 and N2 positions of guanine.

Removal of the sugar residue from the gaunosine product by acid hydrolysis resulted in the corresponding guanine derivative which was also made by reacting guanine with reduced misonidazole. In aqueous solution at pH 11, the guanine product was quantitatively converted to guanine within 20 min. A number of N1-substituted 2-nitroimidazoles and 2-nitroimidazole reacted with guanosine in an analogous manner, giving rise the same product as misonidazole indicating that the C4-C5 fragment from the imidazoles is involved in the modification. Neither misonidazole nor its amine derivative reacted with guanosine. Reduced misonidazole reacted with N^2-methyl guanosine whereas with N1-methylguanosine, reaction was not detected. These reactions provide a molecular mechanism for the cytotoxic and neurotoxic properties of 2-nitroimidazoles.

Formation and Persistence of 3,3′-Dichlorobenzidine-DNA Adducts in Target and Nontarget Tissues of the Rat. D. L. TULLIS and H. C. SIKKA, *Syracuse Research Corp., Syracuse, NY 13210.*

In order to elucidate the role of 3,3′-dichlorobenzidine (DCB)-DNA adducts in carcinogenesis, male Sprague-Dawley rats were given an oral dose of [^{14}C]DCB and sacrificed at 8, 24, or 168 hr after treatment. DNA from target (liver and kidney) and nontarget (lung) tissue was isolated by hydroxylapatite chromatography and enzymatically hydrolyzed to mononucleosides which were then separated by HPLC. At the 8 hr time point, there were no significant differences in total binding among the three tissues; however, there were interesting differences in the loss of bound radioactivity from the DNA of target and nontarget tissues. In the liver, 40% of the bound radioactivity was lost between 8 and 24 hr. Afterwards, the loss was less severe, with 50% of the original amount remaining after 7 days. The kidney followed a similar pattern, with approximately 40% of the bound radioactivity remaining after 7 days; however, the bound radioactivity had been completely lost from lung DNA by 7 days. In the liver and kidney, HPLC analysis of the DNA hydrolyzate revealed five labeled peaks, accounting for 6, 17, 8, 47, and 23% of the adducts, respectively, in the kidney and 2, 4, 21, 22, and 50% in the liver. In both tissues, the first three adducts were not detected after 168 hr, while 30-40% of adducts 4 and 5 remained. In the lung, the respective peaks accounted for 2, 11, 8, 69, and 10% of the adducts; however, by 7 days none of the adducts remained in detectable amounts. There are, thus, two major differences between the adducts formed in target and nontarget tissues. First, adduct 5 is a major product in target, but not in nontarget tissues. Second, loss of adducts 4 and 5 is incomplete in target but not in nontarget tissues. This implies that DCB forms adducts in at least two distinct regions of the cellular DNA, only one of which is accessible to repair systems in target tissues.

^{32}P-Fingerprinting Method for Detection of Bulky Hydrophobic Carcinogen-DNA Adducts. RAMESH C. GUPTA and KURT RANDERATH, *Department of Pharmacology, Baylor College of Medicine, Houston, TX 77030.*

A newly developed ^{32}P-fingerprinting technique for the detection of carcinogen-DNA adducts was modified and applied to the analysis of DNA reacted with active metabolites of 2-acetylaminofluorene and benzo(a)pyrene, respectively. DNA was enzymatically digested to deoxynucleoside 3′-monophosphates which were then converted to [^{32}P]-deoxynucleoside 3′,5′-bisphosphates by T_4 polynucleotide kinase-catalyzed phosphorylation. The hydrophobic adduct nucleotides were found to be retained at the origin of the chromatogram upon polyethyleneimine (PEI)-cellulose TLC in aqueous electrolyte solutions, but to migrate as distinct spots in solvents containing 5 - 8.5 *M* urea. Advantage was taken of this observation to remove ^{32}P-labeled normal nucleotides from adduct nucleotides. This purification enabled us to detect a single adduct in 10^6-10^7 DNA nucleotides. The new method should be applicable to the detection of a large number of carcinogen-DNA adducts of diverse structure without requiring radioactive carcinogens or specific antibodies.

Detection and Quantification of the Reaction Products of 2-Acetylaminofluorene with DNA by a High-Sensitivity Enzyme-Linked Immunosorbent Assay (HS-ELISA). C. J. VAN DER LAKEN, E. KRIEK, G. HERMSEN and M. WELLING, *Division of Chemical Carcinogenesis, Antoni van Leeuwenhoek-Huis, The Netherlands Cancer Institute, 121 Plesmanlaan, 1066 CX Amsterdam, The Netherlands.*

A high-sensitive enzyme-linked immunosorbent assay (HS-ELISA) for the quantification of carcinogen-modified DNA has been developed which makes possible the detection of femtomolar amounts of adduct in a few micrograms of DNA. Fixed amounts of AAF-modified DNA are coated to microtitre plates, DNA samples of known AAF modification serving as standards. Following preincubation with nonimmune serum, antibodies raised in rabbits against bovine serum albumin Guo-8-AAF conjugate are allowed to bind to the modified DNA. As second antibody goat anti-rabbit IgG alkaline phosphatase conjugate is used. The amount of enzyme, directly correlated to the amount of dGuo-8-AAF present in DNA, is determined by the phosphatase catalyzed formation of fluorescent 4-methylumbelliferone from the nonfluorescent substrate 4-methylumbelliferyl phosphate. Representative dose-response curves show a linear relationship between the relative fluorescence and the amount of dGuo-8-AAF from 0.1 fmole to 20 fmole adduct per 0.8 μg of DNA. This method allows the detection of dGuo-8-AAF in DNA at a level of 0.1 μmole adduct per mole DNA-P (one modification per 10^7 deoxynucleotides). The method is 10 times more sensitive than the ultrasensitive enzymatic radioimmunoassay (USERIA) employed by Hsu et al.

Conformational Properties of Adducts between Double-Helical DNA Fragments and 2-Acetylaminofluorene. S. A. ISLAM, S. NEIDLE and A. SUBBIAH, *Cancer Research Campaign, Biomolecular Structure Research Group, King's College, London WC2B 5RL, U.K.*

X-ray crystallographic analyses of three hydroxyl derivatives of 2-acetylaminofluorene have revealed restricted conformational freedom about the acetylamino side chain. Using a combined computer graphics and potential-energy calculation approach, we have employed this information to investigate the low-energy structures of DNA adducts.

Base Displacement in 2-Acetylaminofluorene and 1-Aminofluorene Modified dCpdG; *syn* and *anti* Guanine. S. BROYDE, *Biology Department, New York University, New York, NY 10003* and B. HINGERTY, *Health and Research Safety Division, Oak Ridge National Laboratory, Oak Ridge, TN 37830.*

Minimized conformational potential energy calculations have been performed for the major adduct of *N*-2-acetylaminofluorene (AAF) and *N*-2-aminofluorene (AF) with the deoxydinucleoside monophosphate dCpdG. The DNA backbone conformations that produce base displacement, about which no information was previously available, were ascertained. For the AAF adduct five different combinations of the DNA backbone torsions ω',ω,ψ produce low energy conformers in which the fluorene is coplanar with cytidine, and the guanine is *syn* and twisted nearly perpendicular to the AAF. For the deacetylated AF adduct, three additional backbone types yielded fluorene-cytidine stacked conformers. In these conformers the guanine was *anti*. In addition, important low energy conformers were computed for both adducts which maintain guanine-cytidine stacking. Included are a Z helix for both AAF and AF, and a B helix for AF. Molecular models which show how the dimeric subunits fit into larger structures are presented. The various low energy conformations may occur under differing circumstances, such as extent of modification, base sequence, salt concentration and the nature of the adduct (acetylated or deacetylated).

Force Field Conformational Analysis of Arylamine- and Arylamide-Substituted Deoxyguanosine. K. B. LIPKOWITZ, T. CHEVALIER, M. WIDDIFIELD, *Department of Chemistry, Indiana-Purdue University, 1125 E 38th St., Indianapolis, IN 46205* and W. B. MELCHIOR and F. A. BELAND, *National Center for Toxicological Research, Jefferson AR 72079.*

N-Hydroxy-2-aminofluorene and *N*-hydroxy-4-aminobiphenyl react with DNA to give C8-deoxyguanosine substituted adducts as the major products. The aminofluorene adduct has been reported to be much more efficient than the aminobiphenyl lesion in inducing frameshift mutations in *Salmonella typhimurium* TA 1538. In an attempt to explain the differences in mutagenic efficiency, the conformations of these two adducts were examined with Allinger's force field. For comparison, the *N*-acetylated adducts, as well as deoxyguanosine and 8-aminodeoxyguanosine, were also studied. The calculations indicate that deoxyguanosine exists as a mixture of *syn* and *anti* forms and that the *syn* form is slightly favored for 8-aminodeoxyguanosine. Although the *syn* conformation is also preferred for both *N*-(deoxyguanosin-8-yl)-2-aminofluorene and *N*-(deoxyguanosin-8-yl)-4-aminobiphenyl, the small energy difference between the *syn* and *anti* conformers and the low interconversion barrier indicate that the *anti* form is accessible. The major differences between the aminobiphenyl and aminofluorene C8-deoxyguanosine adducts are that the biphenyl adduct has a lower barrier to rotation and a smaller energy difference between the *syn* and *anti* forms. Whereas the arylamine adducts can exist in *syn* and *anti* conformations, the calculations for the acetylated analogues indicate that *N*-(deoxyguanosin-8-yl)-2-acetylaminofluorene exists only in the *syn* conformation while *N*-(deoxyguanosin-8-yl)-4-acetylaminobiphenyl preferentially adopts the *anti* form.

Conformation of *N*-(Deoxyguanosin-8-yl)-2-acetylaminofluorene Differs in Protein-Free DNA and Chromatin. J. ROSS, G. METZGER and H. WERBIN, *Programs in Biology, University of Texas at Dallas, Richardson, TX 75080.*

Modification of protein-free DNA with *N*-2-acetoxy-*N*-2-acetylaminofluorene results in the formation of *N*-(deoxyguanosin-8-yl)-2-acetylaminofluorene (C-8) adducts in which the fluorene ring inserts between the bases and the guanosine is shifted to the outside of the helix creating localized regions of denaturation. The following data for modified chromatin are inconsistent with this model and lead us to propose the existence of different conformations for C-8 adducts in chromatin and protein-free DNA. Fluorenyl residues in modified chromatin and core particles gave rise to a less intense circular dichroism signal than those in purified DNA: ~-290° vs. ~-180°-cm²/nmole of fluorene residues, respectively. Levels of modification from 1.37 to 2.87 fluorenyl residues per 100 nucleotides that produced a decrease of the melting temperature from 0.8 to 2°C in protein-free DNA had no effect upon the melting temperature of core particles. Adducts were not removed from modified core particles and chromatin by *Neurospora crassa* endonuclease as they were from modified DNA, even though this enzyme at the same concentration (12 units/A_{260} unit) recognized single-stranded regions introduced into the core particles by mild-DNase I digestion. These data demonstrate minimal interaction of the fluorenyl residues with the DNA helix and provide no evidence for their induction of locally denatured regions.

***N*-Acetoxy-*N*-acetylaminofluorene Crosslinks Protein to DNA via a Free-Radical Mechanism.** M. RAYSHELL, J. ROSS and H. WERBIN, *Programs in Biology, University of Texas at Dallas, Richardson, TX 75080.*

The synthetic carcinogen, *N*-acetoxy-*N*-2-acetylaminofluorene (N-AcO-AAF) is known to crosslink protein to DNA when it is added to either cells in culture or to chromatin. Until now the mechanism has remained obscure. To mimic chromatin, a complex of lysozyme and ^{3}H-labeled plasmid DNA (pBR322) was used. A method was devised to quantitate the amount of crosslinking by chromatographing the reacted complex on CM Sephadex 25. Unreacted DNA eluted early and the ^{3}H-crosslinked material was freed from the column with Na_2CO_3. With this method, the following observations were made. There was increasing crosslinking as the amount of carcinogen added to the complex was increased. There was an inverse relation between the amount of crosslinking and the amount of nitrosobenzene (spin trap) added to the reaction mixture. The same observation was made with the stable radical α,α-diphenyl-β-picrylhydrazyl. Acrylamide was polymerized by N-AcO-AAF, and N-AcO-AAF added to DNA containing ^{3}H[CH_3]-labeled thymidine gave rise to ^{3}HOH. These observations are consistent with a free-radical mechanism for the crosslinking, a type of DNA lesion that cannot be repaired efficiently in cells. This idea receives support from the findings that γ-irradiation and ultraviolet radiation crosslink protein to DNA by free-radical mechanisms. It is very likely that many monofunctional carcinogens that crosslink DNA to protein do so via free radicals.

The Hepatocarcinogen Quinoline Does Not Stimulate Microsomal Styrene Oxide Hydrolase, Whereas the Noncarcinogen Isoquinoline Does Stimulate It. M. J. GRIFFIN and A. D. VAZ, *Oklahoma Medical Research Foundation, Oklahoma City, OK 73104.*

Quinoline (Q) in diet (0.10%) when fed for 16 weeks to male Sprague-Dawley rats has been reported to produce liver tumors in 75% of the animals. We fed 16 male Sprague-Dawley rats diet containing 0.15% isoquinoline (IQ) for 16 weeks and found no tumors or hyperplastic nodules at 12, 16 or 28 weeks. A few areas of gamma-GT staining were the only histologic changes noted in the IQ-treated animal livers after 16 weeks. We have previously reported that IQ but not Q stimulates microsomal styrene oxide hydrolase activity *in vitro*, a stimulatory trait shared by the structural analog of IQ, ellipticine. Ellipticine (μM) has been reported to inhibit P-450b. IQ induced (electroimmunoassay) P-450b 2.0-fold, P-450 reductase 1.4-fold and hydrolase 1.9-fold by 14 days. Another study reported microsome-mediated *in vitro* binding of Q to nucleic acid was reduced by P-450 inhibitors, but not by hydrolase inhibitors. The above data suggest that it is IQ's ability to inhibit P-450b which activates it to the *N*-oxide that serves as a major protective mechanism. IQ may induce P-450c (ring epoxidation), explaining the induction of hydrolase. Trp-P-1 and Trp-P-2 were compared as hydrolase stimulators, and again the poorer carcinogen Trp-P-1 was the better stimulator. This postulated mechanism predicts that IQ inhibits P-450b but Q does not, that Q-2,3 epoxide and IE-3,4 epoxide hydrolase are stimulated by IQ, but not Q and that IQ will induce more P-450c than Q. The Q and IQ comparison appears to be an interesting situation in which the parent compound itself regulates its own metabolism, which in turn determines its biologic potency.

Identification of the Major DNA Adduct in Rat Mammary Gland After *in vivo* Administration of the Carcinogen, *N*-Hydroxy-*N*-formyl-2-aminofluorene. W. T. ALLABEN and C. C. WEIS, *National Center for Toxicological Research, Jefferson, AR 72079.*

N-Hydroxy-*N*-formyl-2-aminofluorene (NF) is a mammary gland carcinogen in female Sprague-Dawley rats. Two arylhydroxamic acid *N,O*-acyltransferases have been isolated from mammary gland tissue. One is specific for NF and has been shown to produce tRNA adducts upon incubation with this compound. Moreover, this enzyme may play a major role in the metabolic activation of this compound *in vivo* to a reactive species, since other enzymatic pathways thought to be important in activating aromatic amines are apparently limited, e.g., this tissue does not have detectable levels of PAPS-sulfotransferase or deacylase enzymes. Radiolabeled NF was administered to female Sprague-Dawley rats and the animals were killed at 4 hr, 24 hr and 4 weeks after injection. DNA was then isolated by solvent extraction and hydroxyapatite chromatography. After enzymatic hydrolysis to mononucleosides, DNA adducts were isolated and analyzed by high pressure liquid chromatography. One major adduct was detected and it was chromatographically identical to *N*-(deoxyguanosin-8-yl)-2-aminofluorene (C8-AF). Smaller amounts of its imidazole ring-opened derivative were also identified. The level of the adduct was at maximum 4 hr after dosing and then decreased rapidly by 24 hr. However, after 4 weeks, the amount of adduct had decreased to only about 24% of the 4-hr sample. NF administered to Sprague-Dawley rats results in the induction of fibroadenomas as well as adenocarcinomas, while *N*-hydroxy-*N*-acetyl-2-aminofluorene (NA) produces primarily adenocarcinomas. However, both NF and NA produce the same C8-AF DNA adduct. Since a single C8-AF adduct is formed in mammary gland tissue *in vivo* after NF or NA administration, apparently the nature of this adduct does not explain the difference in tumor response observed with these compounds.

Rapid Decreases in *N*-Hydroxy-2-acetylaminofluorene Sulfotransferase Activity of Liver Cytosols from Rats Fed Carcinogen. DAVID P. RINGER, KIT KAMPSCHMIDT, ROBERT L. KING, JR., STUART JACKSON and DONALD E. KIZER, *The Noble Foundation, Inc., Ardmore, OK 73401.*

In spite of indications that N-OH-AAF sulfotransferase may play a prominent role in initiation and promotion stages of AAF hepatocarcinogenesis, few studies have examined the levels of this enzyme's activity during the course of the carcinogenic process. Employing an assay for N-OH-AAF sulfotransferase described by Mulder et al., we monitored this activating pathway during the initial phase of hepatocarcinogenesis produced by feeding rats a diet containing 0.05% AAF. Our studies revealed an immediate and precipitous decrease (4- to 5-fold) in N-OH-AAF sulfotransferase activity during 5 days of feeding. This activity remained at low levels with continuous feeding of AAF diet throughout 4 weeks, but was shown to be both reversible and AAF-dose-dependent. Parallel monitoring of rat serum GOT activity during AAF-induced decreased sulfotransferase activity failed to indicate appreciable hepatocellular toxicity. Such a rapid and sustained decrease in sulfotransferase activity and the absence of significant hepatocellular toxicity appear inconsistent with its putative role as a cytotoxic pathway responsible for promotion of AAF-initiated carcinogenesis. Other known carcinogens, e.g., aflatoxin, diethylnitrosamine, ethionine, 3'-methyl-4-dimethylaminoazobenzene and thioacetamide, caused equally significant drops in N-OH-AAF sulfotransferase activity by 7 days of feeding, while noncarcinogenic compounds, e.g., *p*-aminoazobenzene, fluorene and sodium barbital, failed to reduce N-OH-AAF sulfotransferase activity. Perhaps decreases in N-OH-AAF sulfotransferase activity can be used as an early indicator of hepatocarcinogenesis.